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TOWNSEND AND TOWNSEND AND CREW, LLP
TWO EMBARCADERO CENTER
EIGHTH FLOOR
SAN FRANCISCO, CA 94111-3834

EXAMINER	
LU, FRANK WEI MIN	
ART UNIT	**PAPER NUMBER**
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/876,374	WILLIAMS ET AL.	
	Examiner	Art Unit	
	Frank W Lu	1634	

-- The MAILING DATE of this communication appears on the cover she t with the correspond nce address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 August 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-66* is/are pending in the application.

4a) Of the above claim(s) *22-48* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4,6-12,15-20,49-51 and 53* is/are rejected.

7)☒ Claim(s) *5,13,14,21,52 and 54-66* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *12/01, 3/02, 1/03*
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Election/Restriction

1. Applicant's election with traverse of species 2, claims 49-66 in the response filed on January 8, 2003 is acknowledged. The traversal is on the ground(s) that "[R]estriction of the invention to species 2 is improper because features found in species number 1 can be found in species number 2. For example, the compounds recited in claim 21 are within the ambit of species 1 and species 2. Moreover, many of the compounds listed in Figure 2 fall within the ambit of both species 1 and species 2. Thus, the restriction to a single species is improper, as for example, claim 21 falls within the ambit of both species.".

After carefully considers applicant's arguments, the examiner agrees to withdraw the restriction requirement mailed on August 20, 2002. Therefore, claims 1-21 and 49-66 will be examined.

Claim Numbers

2. The examiner notes that applicant renumbered claims 49-66 as claims 51-68 in the response filed on January 8, 2003. In fact, in the first restriction requirement sent on July 12, 2002, the examiner clearly indicated that "[N]ote that original application was filed with claims 1-37 and 40-50. Since there is no claims 38 and 39, claims 40-50 have been renumbered, starting at 38, as accorded under Rule 1.126.". Therefore, claims 49-66 do not need to be renumbered again as suggested by applicant.

Specification

3. The disclosure is objected to because of the following informalities: page 1, first paragraph of the specification cites a US Patent without patent number.

Appropriate correction is required.

Claim Objections

4. Claim 58 is objected to because of the following informalities: there should be a coma after "deoxyguanosine triphosphate". Appropriate correction is required.

Claim Rejections - 35 USC § 112

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 6-9 and 20 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Note that claims 7-9 are dependent on claim 6.

7. Claim 6 is rejected as vague and indefinite in view of claims 3 and 6. According to claim 3, said charge-switch NP probe is a nucleotide triphosphate (NTP) while, according to claim 6, said NTP probe is selected from the group consisting of a deoxynucleotide triphosphate (dNTP) and a nucleotide triphosphate (NTP). Since claim 6 is dependent on claim 3, claim 6 must further limit claim 3. However, in view of claims 3 and 6, claim 6 does not further limit claim 3 and it

appears that "NTP" recited in claim 3 and "NTP" recited in claim 6 are identical. Therefore, claim 3 and claim 6 do not correspond each other. Please clarify.

8. Claim 20 is rejected as vague and indefinite. Since claim 1, which claim 20 is dependent on, is directed to a product, claim 20 must be directed to a product. In view of claim 20, it is unclear that claim 20 is directed to a method step or is directed to a product. If claim 20 is directed to a method, it is well established that even though product by process claims are limited by and defined by the process, the determination of the patentability of the product is based on the product itself. *In re Thorpe*, 227 USPQ 964, 966 (Fed. Cir. 1985). Please clarify.

Claim Rejections - 35 USC § 102

9. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
> (e) the invention was described in-
> (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effect under this subsection of a national application published under section 122(b) only if the international application designating the United States was published under Article 21(2)(a) of such treaty in the English language; or
> (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that a patent shall not be deemed filed in the United States for the purposes of this subsection based on the filing of an international application filed under the treaty defined in section 351(a).
> (f) he did not himself invent the subject matter sought to be patented.

10. Claims 1, 2, 10, 19, and 20, 49-51 and 53 are rejected under 35 U.S.C. 102(b) as being anticipated by Brow *et al.,* (US Patent No. 6,001,567, published on December 14, 1999).

The invention is directed to an intact charge-switch nucleotide phosphate (NP). Claim 1 requires that an intact NP probe having a terminal phosphate with a fluoroscope moiety attached thereto, said intact NP probe having a first molecular charge associated therewith, whereupon cleavage of said terminal phosphate as a phosphate fluorophore moiety, said phosphate fluoroscope moiety carries a second molecular charge, wherein the difference between said first molecular charge and said second molecular charge is at least 0.5. Claim 2 further limit claim 1 and requires that either said intact NP probe has a positive molecular charge, or wherein upon cleavage of said terminal phosphate fluorophore moiety, said terminal phosphate fluorophore moiety carries a molecular positive charge relative to said intact NP probe. Claim 10 further limits claim 10 and requires that said fluorophore moiety is a member selected from the group consisting of fluorescein, 5-carboxyfluorescein (FAM), rhodamine, 5-(2'-aminoethyl) aminonapthalene-l-sulfonic acid (EDANS), anthranilamide, coumarin, terbium chelate derivatives, Reactive Red 4, BODIPY dyes and cyanine dyes. Claim 19 further limits claim 1 and requires that the difference between said first molecular charge and said second molecular charge is from 0.5 to 4.0. Claim 20 requires that the difference between said first molecular charge and said second molecular charge is at least 0.5 in pure water at pH 7.0. Claim 49 requires, that upon enzymatic cleavage of an intact charge-switch NP probe to produce a phosphate detectable moiety, said phosphate detectable moiety migrates to an electrode, and said intact charge-switch NP probe migrates to the other electrode. "charge-switch nucleotide" is defined as "a labeled nucleotide phosphate (e.g., γ-NP-Dye) that upon release or cleavage of a phosphate detectable moiety (e.g., PPi-Dye) has a different net charge associated with the cleavage product compared to the intact

nucleotide phosphate probe (e.g., γ- NP-Dye). In certain preferred aspects, the attachment of the dye to the PPi is via a nitrogen in lieu of an oxygen. Preferably, the charge difference between the intact γ-NP-Dye and the PPi-Dye is at least 0.5, and more preferably about 1 to about 4" (see the specification, page 7, last paragraph bridging to page 8, first paragraph). "phosphate detectable moiety" is defined as "a detectable cleavage product from a NP probe of the present invention. Examples include, but are not limited to, PPi-Dye, PP-F, P-Dye, a phosphate fluoroscope moiety, a terminal phosphate fluoroscope moiety, a detectable moiety, charged groups, electrically active groups, detectable groups, reporter groups, combinations thereof, and the like." (see the specification, page 8, third paragraph). Claim 50 further limits claim 49 and requires that said intact NP probe either has a positive molecular charge or, upon cleavage of said phosphate detectable moiety, said phosphate detectable moiety carries a different charge relative to said intact NP probe. Claim 51 further limits claim 49 and requires that said intact NP probe either has a negative molecular charge or, upon cleavage of said phosphate detectable moiety, said phosphate detectable moiety carries a different charge relative to said intact NP probe. Claim 53 further limits claim 49 and requires that said intact NTP probe has a positive charge.

Brow *et al.,* teach detection of nucleic acid sequences by invader-directed cleavage. A modified oligonucleotide 61 (5'-Cy3-AminoT-Amino-TCTTTTCACCAGCGAGAC GGG-3') carried a net negative charge. After cleavage with a cleavage enzyme, the following oligonucleotides were generated: 5'-CTTTTCACCAGCGAGACGGG-3' (residues 3-22 of SEQ ID NO:61) and 5'-Cy3-AminoT-Amino-T-3'. 5'-Cy3-AminoT-Amino-T-3' bore a detectable moiety (the positively-charged Cy3 dye) and two amino-modified bases. Since, in the

5'-Cy3-AminoT-Amino-T-3'oligonucleotide, the amino-modified bases and the Cy3 dye contributed positive charges in excess of the negative charges contributed by the phosphate groups, this oligonucleotide had a net positive charge. The other, longer cleavage fragment bore a net negative charge (see lines 39-65 in column 22, lines 50-67 in column 97, and lines 1-28 in column 98). As shown in Figure 56 and Example 23, compounds 70 or 74 contained two amino modified thymidines that, under reaction conditions, displayed positively charged R-NH_3^+ groups attached at the C5 position through a C_{10} or C_6 linker, respectively. The compound 70 or 74 possessed a Cy-3 dye positioned at the 5'-end which individually was positively charged under reaction and isolation conditions described in this example. Because compounds 70 or 74 were 3'-end phosphorylated, they consisted of four negative charges and three positive charges. For the simplicity of analysis, each group was assigned a whole number of charges, although it was realized that, depending on the pKa of each chemical group and ambient pH, a real charge might differ from the whole number assigned. It was assumed that this difference was not significant over the range of pHs used in the enzymatic reactions studied here (see Figure 56 and lines 12-41 of column 96).

Regarding claims 49-51, charge-switch nucleotide is defined as a labeled nucleotide phosphate (e.g., γ-NP-Dye) that upon release or cleavage of a phosphate detectable moiety (e.g., PPi-Dye) has a different net charge associated with the cleavage product compared to the intact nucleotide phosphate probe (e.g., γ- NP-Dye) wherein a labeled nucleotide phosphate, a phosphate detectable moiety, and the intact nucleotide phosphate probe are not limited to γ-NP-Dye, Ppi-Dye, and γ- NP-Dye respectively while phosphate detectable moiety is defined as

a detectable cleavage product from a NP probe but are not limited to, PPi-Dye, PP-F, P-Dye, a phosphate fluoroscope moiety, a terminal phosphate fluoroscope moiety, a detectable moiety, charged groups, electrically active groups, detectable groups, reporter groups, and combinations thereof. Since it is generally accepted that oligonucleotide is a probe, modified oligonucleotide 61 is a probe. Since the modified oligonucleotide 61 bears a net negative charge and the modified oligonucleotide 61, upon enzymatic cleavage (ie., a cleavage enzyme), releases a shorter cleavage fragment 5'-Cy3-AminoT-Amino-T-3' having a net positive charge, the modified oligonucleotide 61 is a NP probe and 5'-Cy3-AminoT-Amino-T-3' is a phosphate detectable moiety since it carries the phosphate groups and a fluorescence dye (ie., Cy3) wherein said phosphate detectable moiety (ie., 5'-Cy3-AminoT-Amino-T-3') carries a different charge relative to said intact NP probe (ie., the modified oligonucleotide 61) as recited in claims 50 and 51. Since it is known that an oligonucleotide having a net positive charge migrates toward the negative electrode in an electrical field while an oligonucleotide having a net negative charge migrates toward the positive electrode in an electrical field (see lines 6-21 in column 23), the NP probe (ie., modified oligonucleotide 61) migrates toward the positive electrode in an electrical field while the phosphate detectable moiety (ie., 5'-Cy3-AminoT-Amino-T-3') migrates toward the negative electrode in an electrical field as recited in claim 49. Although claims 50 and 53 require that said intact NP probe has a positive charge while Brow *et al.,* disclose that intact NP probe (ie., the modified oligonucleotide 61) has positive charges, since "has" of claims 49 and 53 is considered as "comprising", claims 50 and 53 do not limit that said intact NP probe has only one positive charge, claims 50 and 52 are anticipated by Brow *et al.*.

Regarding claims 1, 2, 10, and 19, since the Cy-3 dye of the compound 70 or 74 is attached to a phosphate group positioned at the 5'-end of the compound (see Figure 58), the phosphate group positioned at the 5'-end of the compound is a terminal phosphate with a fluorophore moiety attached thereto as recited in claim 1. Since, if the compound 76 is cleaved on a 3', 5'-phosphodiester bond which is related to the first phosphate (for the location of cleaved 3', 5'-phosphodiester bond, see arrow in attached Figure 58), the phosphate group positioned at the 5'-end of the compound (ie., a terminal phosphate with a fluorophore moiety attached thereto) is released from the compound 76 and the released compound is a phosphate fluorophore moiety as recited in claim 1 since the released compound has a fluorophore moiety. Since the released compound consists of two negative charge and a positive charges and has net charge -1 (see attached Figure 58) while the compound 76 consists of four negative charges and two positive charges and has net charge -2, the compound 76 and the released compound are an intact NP probe and a phosphate fluorophore moiety respectively wherein the difference between said first molecular charge (ie., -2) and said second molecular charge(ie., -1) is at least 0.5 [ie., 1] as recited in claims 1 and 19. Since the compound 76 consists of four negative charges and two positive charges, said intact NP probe (ie., the compound 76) has a positive molecular charge as recited in claim 2. Since it is known that cy3 is one of cyanine dyes, claim 10 is anticipated by Brow *et al.*.

Regarding claim 20, since Brow *et al.,* indicates that charge difference is not significant over the range of pHs used in the enzymatic reactions studied here (see column 96, lines 12-42) and it is known that pH 7.0 is a pH near the pHs of the enzymatic reactions studied here, charge difference between the intact NP probe and the phosphate fluorophore moiety in pH 7.0 should

not significantly different from charge difference between the intact NP probe and the phosphate fluorophore moiety in the pHs of the enzymatic reactions taught by Brow *et al.*. Therefore, claim 20 is anticipated by Brow *et al.*.

Therefore, Brow *et al.*, teach all limitations recited in claims 1, 2, 10, 19, and 20, 49-51 and 53.

11. Claims 1-4, 6-12, and 15-20 are rejected under 35 U.S.C. 102(e) as being anticipated by Williams *et al.*, (US Patent No. 6,232,075 B1, filed on December 13, 1999, priority date: December 14, 1998).

The invention is directed to an intact charge-switch nucleotide phosphate (NP) probe. Claim 1 requires that an intact NP probe having a terminal phosphate with a fluoroscope moiety attached thereto, said intact NP probe having a first molecular charge associated therewith, whereupon cleavage of said terminal phosphate as a phosphate fluorophore moiety, said phosphate fluoroscope moiety carries a second molecular charge, wherein the difference between said first molecular charge and said second molecular charge is at least 0.5. Claim 2 further limit claim 1 and requires that either said intact NP probe has a positive molecular charge, or wherein upon cleavage of said terminal phosphate fluorophore moiety, said terminal phosphate fluorophore moiety carries a molecular positive charge relative to said intact NP probe. Claim 3 further limits claim 1 and requires that said charge-switch NP probe is a nucleotide triphosphate wherein said terminal phosphate is a pyrophosphate with a fluorophore moiety attached thereto. Claim 4 further limits claim 3 and requires that said intact NTP probe has a positive charge.

Claim 6 further limit claim 3 and requires that said NTP probe is dNTP or NTP. Claim 7 further limits claim 6 and requires that said NTP probe is dNTP. Claim 8 further limits claim 7 and requires that dNTP is a member selected from the group consisting of deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate, deoxythymidine triphosphate and deoxyuridine triphosphate. Claim 9 further limits claim 6 and requires that NTP is a member selected from the group consisting of adenosine triphosphate, cytosine triphosphate, guanosine triphosphate and uridine triphosphate. Claim 10 further limits claim 10 and requires that said fluorophore moiety is a member selected from the group consisting of fluorescein, 5-carboxyfluorescein (FAM), rhodamine, 5-(2'-aminoethyl) aminonapthalene-l-sulfonic acid (EDANS), anthranilamide, coumarin, terbium chelate derivatives, Reactive Red 4, BODIPY dyes and cyanine dyes. Claim 11 further limits claim 3 and requires that said fluorophore moiety is attached to said terminal phosphate via a linker. Claim 12 further limits claim 11 and requires that said fluorophore linker is an alkylene group having between about 5 to about 12 carbons. Claim 15 further limits claim 1 and requires that at least one of the phosphate moieties of said nucleotide phosphate probe has an ionized oxygen atom with a counter-cation associated therewith. Claim 16 further limit the counter-cation recited in claim 15 and requires that said counter-cation is a metal ion. Claim 17 further limits the metal ion recited in claim 16 and requires that said metal ion is selected from the group consisting of Mg^{++}, Mn^{++}, K^{+} and Na^{+}. Claim 18 further limits claim 11 and requires that wherein said fluorophore moiety is BODIPY TR. Claim 19 further limits claim 1 and requires that the difference between said first molecular charge and said second molecular charge is from 0.5 to 4.0. Claim 20 requires that the difference between

said first molecular charge and said second molecular charge is at least 0.5 in pure water at pH 7.0.

Williams *et al.,* teach heterogeneous assay for pyrophosphate detection. Figure 4 showed a nucleotide triphosphate (NTP) probe comprising a dNTP having a γ phosphate with a fluorophore moiety attached thereto and a quencher moiety sufficiently proximal to the fluorophore moiety to prevent fluorescence of the fluorophore moiety (see Figure 4, and columns 3 and 4). As illustrated in Figure 1A, dNTP incorporation into a growing oligonucleotide by a DNA polymerase such as T7 DNA polymerase resulted in pyrophosphate cleavage. In this reaction, the phosphate ester bond between the α and β phosphates of the incorporated nucleotide was cleaved by the DNA polymerase, and the β-γ-diphosphate (pyrophosphate) was released in solution. In the situation wherein a fluorophore was attached to the γ phosphate of the dNTP, the fluorophore was released from the nucleotide along with the pyrophosphate group (see Figures 1A, 1B, and 2C, and lines 23-63 of column 6).

Regarding claims 1, 15-17, 19, and 20, since the γ phosphate of the dNTP in Figure 4 is attached to a fluorophore, the dNTP taught by Williams *et al.,* is a NP probe having a terminal phosphate with a fluoroscope moiety as recited in claim 1. As taught by Williams *et al.,* dNTP incorporation into a growing oligonucleotide by a DNA polymerase results in pyrophosphate cleavage. Since in example 3, DABCYL-dUTP or DABCYL-dUTP-thiol or DABCYL-dUTP-BODIPY TR is stored in a buffer at pH 7 (ie.,Buffers A and B) after they are purified from a reversed-phase HPLC (see column 18, lines 61-67 and column 19, lines 1-29) and one of DNA polymerases used by Williams *et al.,* is T7 DNA polymerase (see column 6, lines 23-

36 and column 18, lines 1-9), and it is known that pH for reaction buffer of T7 DNA polymerase is 7.5 (see the attachment for T7 DNA polymerase), dNTP taught by Williams *et al.*, is in either a buffer with a pH 7 or a buffer with pH 7.5. Since various different fluorescence dyes such as cy3 or cy5 are used as the fluorophore moiety and the quencher moiety in fluorescence labeled dNTP (see columns 12 and 13), **here we assumes that, in dNTP in Figure 4, its fluorophore moiety do not bear any charge and its quencher moiety is cy3**. Since it is known that cy3 is a fluorescence dye carrying a net single positive charge (see page 2, last paragraph of the attachment for CyDye) and dNTP in Figure 4 of Williams *et al.,* has three negative charges (see Figure 4) while the bases in dNTP are mostly uncharged at pH from about 6.5 to about 8.5 (see the specification, page 11, third paragraph), under an ideal condition (without considering effects of a buffer), net charge of the dNTP in Figure 4 of Williams *et al.,* is -2. Since the fluorophore moiety can be released from the dNTP along with the pyrophosphate group after the cleavage (see Figure 2) and the fluorophore moiety do not bear any charge, under an ideal condition (without considering effects of a buffer), net charge of pyrophosphate (PPi)-the fluorophore moiety released from the dNTP in Figure 4 of Williams *et al.,* is -3 (with three negative charges, for PPi structure, see Figure 1 of the specification). Therefore, under an ideal condition (without considering effects of a buffer), dNTP in Figure 4 of Williams *et al.,* which has a γ phosphate with a fluorophore moiety that does not bear any charge and has cy3 quencher moiety, is an intact NP probe having a terminal phosphate with a fluoroscope moiety attached thereto, said intact NP probe having a first molecular charge associated therewith (ie., -2), whereupon cleavage of said terminal phosphate as a phosphate fluorophore moiety (PPi-fluorophore moiety), said

phosphate fluoroscope moiety carries a second molecular charge (ie.,-3), wherein the difference between said first molecular charge (ie., -2 in the dNTP in Figure 4 wherein the fluorophore moiety do not bear any charge and the quencher moiety is cy3) and said second molecular charge (ie., -3 in PPi- fluorophore moiety) is at least 0.5 (ie., 1) as recited in claims 1 and 19. Since it is known that pyrophosphate (PPi) released from dNTP loses 0.26 unit of negative charge in pure water at pH 7 due to hydrogen ion equilibration with the terminal phosphate oxygen (see the specification, page 11, last paragraph and page 12, first paragraph), net charge of pyrophosphate (PPi)-the fluorophore moiety released from the dNTP in Figure 4 of Williams *et al.,* becomes -2.74 [-3 +(+0.26)=-2.74] at pH 7 instead of -3 under a ideal condition (without considering effects of a buffer). Therefore, the difference between said first molecular charge (ie., -2 in the dNTP in Figure 4 wherein the fluorophore moiety do not bear any charge and the quencher moiety is cy3) and said second molecular charge (ie., -2.74 in ppi- fluorophore moiety) in pure water pH 7 is at least 0.5 (ie., 0.74) as recited in claims 1, 19, and 20. Since it is known that T7 DNA polymerase buffer contains Mg^{2+} (ie., 10 mM $MgCl_2$, see the attachment related to T7 DNA polymerase) and Mg^{2+} binds to the terminal phosphate (ie. γ-phosphate) to neutralize negative charges of both dNTP and cleavage pyrophosphate (see the specification, second paragraph of page 12 and last paragraph of page 13), in the T7 DNA polymerase buffer, net charge of dNTP in Figure 4 of Williams *et al.,* and pyrophosphate (PPi)-the fluorophore moiety released from the dNTP in Figure 4 of Williams *et al.,* becomes 0 [-2+(+2)=0] and -1 [-3 +(+2)=-1] respectively. Therefore, the difference between said first molecular charge (ie., 0 in the dNTP in Figure 4 wherein the fluorophore moiety do not bear any charge and the quencher moiety is cy3) and said

second molecular charge (ie., -1 in PPi- fluorophore moiety) in T4 polymerase buffer is at least 0.5 (ie., 1) as recited in claims 1 and 15-17. According to the definition, "terminal phosphate oxygen" is "the secondary ionization oxygen atom (PK ~6.5) attached to the terminal phosphate atom in a nucleotide phosphate probe.". Since Mg^{2+} in the T7 DNA polymerase buffer binds to the terminal phosphate (ie., γ-phosphate) and the secondary ionization oxygen atom attached to the terminal phosphate atom, a counter-cation (ie., Mg^{2+}) is indirectly associated with an ionized oxygen (ie., the secondary ionization oxygen atom is attached to the terminal phosphate atom) as recited in claims 15-17.

Regarding claims 2-4, 6-12, and 18, since the fluorophore is released from the dNTP in Figure 4 of Williams *et al.,* along with the pyrophosphate group after the cleavage, the intact NP probe (ie., dNTP in Figure 4 of Williams *et al.,*) is a nucleotide triphosphate wherein the PPi-fluorophore moiety is the terminal phosphate as recited in claim 3. Since the quencher moiety of dNTP in Figure 4 is cy3 that carries a net single positive charge, the intact NP probe (ie., dNTP in Figure 4 of Williams *et al.,*) has a positive molecular charge as recited in claims 2 and 4. Since NTP probe taught by Williams *et al.,* can be dATP, dCTP, dGTP, dTTP, dUTP, ATP, CTP, GTP, and UTP, claims 6-9 are anticipated by Williams *et al.*. Since fluorophore moiety is attached to γ phosphate of the dNTP via a linker wherein said linker is an alkylene group having between about 5 to about 12 carbons (see Williams *et al.,* column 21, claims 20 and 21), claims 11 and 12 are anticipated by Williams *et al.*. Since various different fluorescence dyes such as BODIPY, FAM, and Texas Red (see column 12, line 44-67, and column 13, lines 1-19) are used

as the fluorophore moiety of dNTP taught by Williams *et al.*, and it is known that Texas Red is also called as BODIPY TR, claims 10 and 18 are anticipated by Williams *et al.*.

Therefore, Williams *et al.,* teach all limitations recited in claims 1-4, 6-12, and 15-20.

12. Claims 1-4, 6-12, and 15-20 are rejected under 35 U.S.C. 102(f) because the applicant did not invent the claimed subject matter.

The patent above teaches all limitations recited in claim 1-4, 6-12, and 15-20. However, there is only one inventor, John G. K. Williams in above patent. In contrast, the inventors of this application includes multiple inventors, John G.K. Williams, Gregory R. Bashford, Jiyan Chen, Dan Draney, Nara Narayanan, Bambi L. Reynolds, and Pamela Sheaff. Since inventors Gregory R. Bashford, Jiyan Chen, Dan Draney, Nara Narayanan, Bambi L. Reynolds, and Pamela Sheaff do not list in above patent and the patent above teaches all limitations recited in claim 1-4, 6-12, and 15-20, these people can not considered as inventors of this instant application.

Double Patenting

13. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686

F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

14. Claims 1-4, 6-12, and 18-20 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 12-28 of U.S. Patent No.6,232,075B1. Although the conflicting claims are not identical, they are not patentably distinct from each other because the examined claims in this instant application is either anticipated by, or would have been obvious over, the reference claims. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

Regarding claim 1-4, 6-12, 19, and 20, claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 teach that a nucleotide triphosphate (ie., dNTP) having a γ-phosphate with a fluorophore moiety attached thereto and a quencher moiety sufficiently proximal to said fluorophore moiety to prevent fluorescence of said fluorophore moiety wherein said quencher moiety is covalently bound to the base of said nucleotide triphosphate (ie., dNTP) and said

quenched moiety is a rhodamine dye (see columns 20 and 21). Since a γ-phosphate of a nucleotide triphosphate (ie., dNTP) is attached to a fluorophore moiety and it is known that γ-phosphate of dNTP is a terminal phosphate, claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 teach an intact NP probe having a terminal phosphate with a fluoroscope moiety attached thereto as recited in claim 1 of this instant application. Since claim 18 of U.S. Patent No.6,232,075B1 shows that said fluorophore moiety can be a rhodamine dye and it is known that one of rhodamine dyes, rhodamine 110, with a net single positive charge (see attachment for rhodamine 110), **here we assumes that, in dNTP recited in claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 , its fluorophore moiety do not bear any charge and its quencher moiety is rhodamine 110.** Since there is no pH in claim 1 of this instant application and claims 1-30 of U.S. Patent No.6,232,075B1, we assumes that dNTP taught in claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 is in either a buffer with a pH 7 or a buffer with pH 7.5 because, as shown in U.S. Patent No.6,232,075B1, DABCYL-dUTP or DABCYL-dUTP-thiol or DABCYL-dUTP-BODIPY TR is stored in a buffer at pH 7 (ie.,Buffers A and B) after they are purified from a reversed-phase HPLC (see column 18, lines 61-67 and column 19, lines 1-29) and one of DNA polymerases used in U.S. Patent No.6,232,075B1 is T7 DNA polymerase (see column 6, lines 23-36 and column 18, lines 1-9) since it is known that pH for reaction buffer of T7 DNA polymerase is 7.5 (see above). Since dNTP recited in claims 12, 13, and 19 of U.S. Patent No.6,232,075B1 has three negative charges contributed by each ionizable oxygen atom on each phosphate of dNTP while the bases in dNTP are mostly uncharged at pH from about 6.5 to about 8.5 (see the specification, page 11, third paragraph), under an ideal condition (without

considering effects of a buffer), net charge of the dNTP recited in claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 is -2. Since the fluorophore moiety can be released from the dNTP recited in claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 along with the pyrophosphate group after the cleavage wherein said fluorophore moiety do not bear any charge, under an ideal condition (without considering effects of a buffer), net charge of pyrophosphate (PPi)-the fluorophore moiety released from the dNTP recited in claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 is -3 (with three negative charges, for PPi structure, see Figure 1 of the specification). Therefore, under an ideal condition (without considering effects of a buffer), dNTP recited in claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 which has a γ phosphate with a fluorophore moiety that does not bear any charge and has rhodamine 110 as a quencher moiety, is an intact NP probe having a terminal phosphate with a fluoroscope moiety attached thereto, said intact NP probe having a first molecular charge associated therewith (ie., -2), whereupon cleavage of said terminal phosphate as a phosphate fluorophore moiety (PPi-fluorophore moiety), said phosphate fluoroscope moiety carries a second molecular charge (ie., -3), wherein the difference between said first molecular charge (ie., -2 in the dNTP recited in claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1) and said second molecular charge (ie., -3 in PPi- fluorophore moiety) is at least 0.5 (ie., 1) as recited in claims 1 and 19 of this instant application. Since it is known that pyrophosphate (PPi) released from dNTP loses 0.26 unit of negative charge in pure water at pH 7 due to hydrogen ion equilibration with the terminal phosphate oxygen (see the specification, page 11, last paragraph and page 12, first paragraph), net charge of pyrophosphate (PPi)-the fluorophore moiety released from the dNTP of claims 12-

15, 18, and 19 of U.S. Patent No.6,232,075B1 becomes -2.74 [-3 +(+0.26)=-2.74] at pH 7.0 instead of -3 under a ideal condition (without considering effects of a buffer). Therefore, the difference between said first molecular charge (ie., -2 in the dNTP recited in claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 wherein the fluorophore moiety do not bear any charge and the quencher moiety is rhodamine 110) and said second molecular charge (ie., -2.74 in PPi-fluorophore moiety) in pure water pH 7 is at least 0.5 (ie., 0.74) as recited in claim 20. Since the fluorophore is released from the dNTP of claims 12-15, 18, and 19 of U.S. Patent No. 6,232,075B1 along with the pyrophosphate group after the cleavage, the dNTP taught by claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 is a nucleotide triphosphate wherein the PPi-fluorophore moiety is the terminal phosphate as recited in claim 3. Since the quencher moiety of dNTP of claims 12-15, 18, and 19 of U.S. Patent No.6,232,075B1 is rhodamine 110 that carries a net single positive charge, dNTP taught in claims 12-15, 18, and 19 of U.S. Patent No. 6,232,075B1 has a positive molecular charge as recited in claims 2 and 4. Note that claims 6-12 of this instant application are identical to claims 14-17 and 19-21 of U.S. Patent No.6,232,075B1.

Regarding claim 18, since claims 19 and 27 of U.S. Patent No.6,232,075B1 teach that said fluorophore moiety is BODIPY dyes and it is known that BODIPY TR is one kind of BODIPY dyes, claims 19 and 27 of U.S. Patent No.6,232,075B1 teach claim 18 of this instant application.

Therefore, although claims 1-4, 6-12, and 18-20 in this instant application are not identical to claims 12-28 of U.S. Patent No.6,232,075B1, claims 12-28 of U.S. Patent No.6,232,075B1 are directed to the same subject matter and fall entirely within the scope of claims 1-4, 6-12, and

18-20 in this instant application. In other words, claims 1-4, 6-12, and 18-20 in this instant application are anticipated by claims 12-28 of U.S. Patent No.6,232,075B1.

Assignee Required

16. As shown above, claims 1-4, 6-12, and 18-20 are directed to an invention not patentably distinct from claims 12-28 of commonly assigned U.S. Patent No.6,232,075B1 (for detail, see above). The Examiner notes that this instant applicant was filed on June 6, 2001. However, an assignment of this instant application was recorded by the office on October 9, 2001, which was four months later than filing date of this instant application. Note that the U.S. Patent and Trademark Office normally will not institute an interference between applications or a patent and an application of common ownership (see MPEP § 2302). Commonly assigned US application 09/876,374 (this instant application) and U.S. Patent No.6,232,075B1, discussed above, would form the basis for a rejection of the noted claims under 35 U.S.C. 103(a) if the commonly assigned case qualifies as prior art under 35 U.S.C. 102(f) or (g) and the conflicting inventions were not commonly owned at the time the invention in this application was made. In order for the examiner to resolve this issue, the assignee is required under 35 U.S.C. 103(c) and 37 CFR 1.78(c) to either show that the conflicting inventions were commonly owned at the time the invention in this application was made or to name the prior inventor of the conflicting subject matter. Failure to comply with this requirement will result in a holding of abandonment of the application.

A showing that the inventions were commonly owned at the time the invention in this

application was made will preclude a rejection under 35 U.S.C. 103(a) based upon the commonly assigned case as a reference under 35 U.S.C. 102(f) or (g), or 35 U.S.C.102(e) for applications filed on or after November 29, 1999.

Conclusion

17. Claims 5, 13, 14, 21, 52, and 54-66 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

18. No claim is allowed.

19. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993)(See 37 CAR § 1.6(d)). The CM Fax Center number is either (703) 308-4242 or (703)305-3014.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Frank Lu, Ph.D., whose telephone number is (703) 305-1270. The examiner can normally be reached on Monday-Friday from 9 A.M. to 5 P.M.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion, can be reached on (703) 308-1119.

Any inquiry of a general nature or relating to the status of this application should be directed to the patent Analyst of the Art Unit, Ms. Chantae Dessau, whose telephone number is (703) 605-1237.

Frank Lu
May 2, 2003

Part of the office action

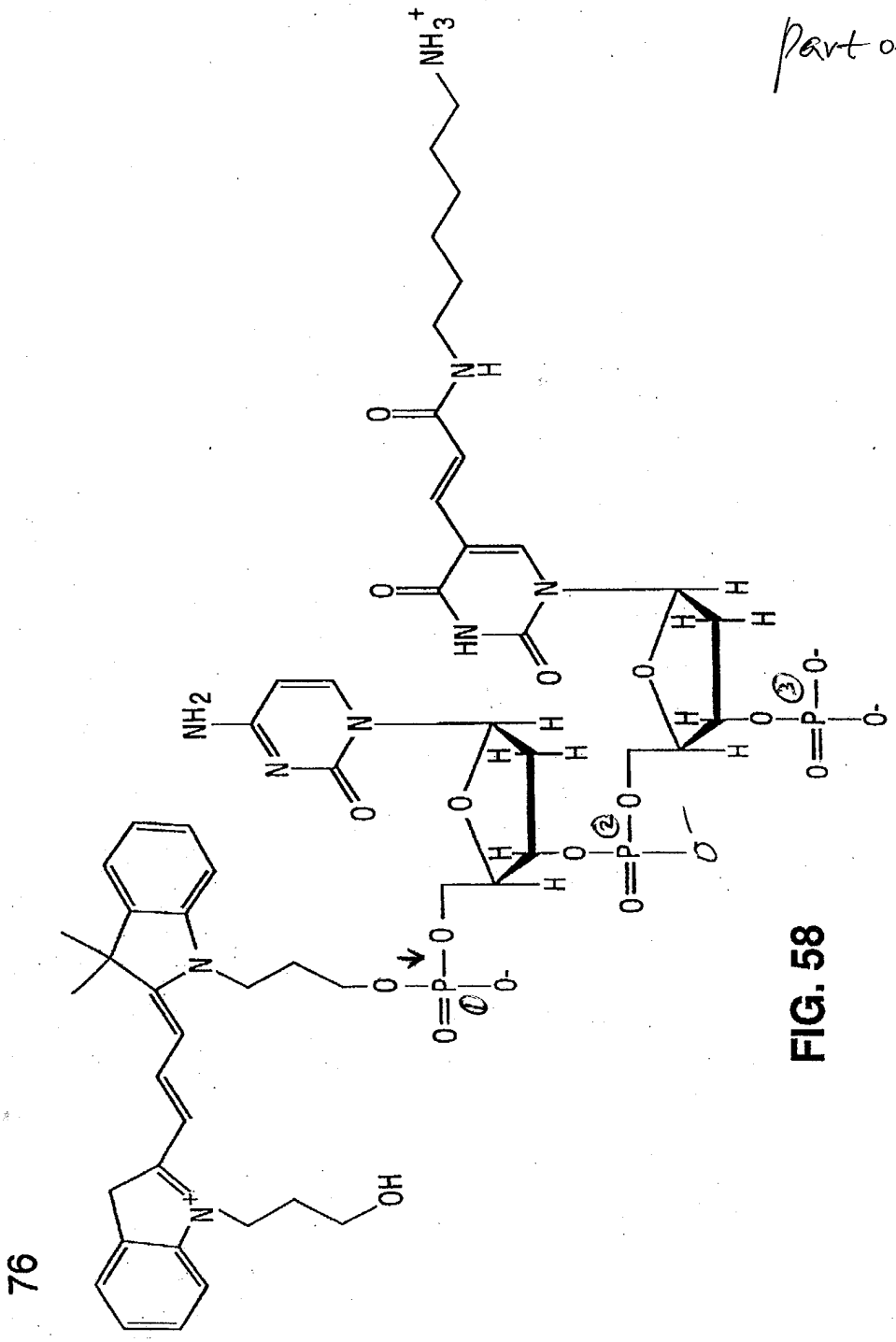

FIG. 58

part of the office action

T7 DNA Polymerase (unmodified) RR

#256S 200 units **$50**
#256L 1000 units **$200**

- *Isolated from a recombinant source*
- *Second strand synthesis in site-directed mutagenesis*
- *Supplied with 10X Reaction Buffer*

Description: T7 DNA polymerase catalyzes the replication of T7 phage DNA during infection. The protein dimer has two catalytic activities: DNA polymerase activity and strong 3′→ 5′ exonuclease (1,2,3). The high fidelity and rapid extension rate of the enzyme make it particularly useful in copying long stretches of DNA template.

Source: T7 DNA Polymerase consists of two subunits: T7 gene 5 protein (84 kilodaltons) and *E. coli* thioredoxin (12 kilodaltons) (1,4–7). Each protein is cloned and overexpressed in a T7 expression system in *E. coli* (4).

Applications:
- Second strand synthesis in site-directed mutagenesis protocols (8).

Reaction Buffer: 1X T7 DNA Polymerase Buffer [20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol]. Supplement with 0.05 mg/ml BSA and dNTPs (not included). Incubate at 37°C.

Notes on Use:
The high polymerization rate of the enzyme makes long incubations unnecessary.

T7 DNA Polymerase is not suitable for DNA sequencing.

Quality Assurance: Purified free of endonuclease contamination.

Unit Definition: One unit is defined as the amount of enzyme required to convert 10 nmole of deoxynucleotides to an acid insoluble form in 30 minutes at 37°C.

Unit Assay Conditions: 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.05 mg/ml BSA, 0.15 mM each dNTP, 0.5 mM heat denatured calf thymus DNA and enzyme.

Concentration: 10,000 units/ml.

Storage Conditions: 50 mM KPO_4 (pH 7.0), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol. Store at –20°C.

References:

(1) Hori, K. et al (1979) *J. Biol. Chem.* 254, 11598-11604.
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(5) Grippo, P. and Richardson, C. C. (1971) *J. Biol. Chem.* 246, 6867-6873.
(6) Modrich, P. and Richardson, C. C. (1975) *J. Biol. Chem.* 250, 5515-5522.
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M-MuLV Reverse Transcriptase RR

#253S 500 units **$50**
#253L 2,500 units **$200**

- *Isolated from a recombinant source*
- *Synthesizes cDNA from single-stranded RNA or DNA*
- *Enzyme of choice for copying long mRNAs*
- *Supplied with 10X Reaction Buffer*

Description: Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase is an RNA-dependent DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using either single-stranded RNA or DNA templates (1,2). M-MuLV Reverse Transcriptase has much lower RNase H activity than AMV Reverse Transcriptase, an important advantage when synthesizing cDNAs from long mRNAs (3). Both M-MuLV and AMV Reverse Transcriptase lack 3′→ 5′ exonuclease function.

Source: Isolated from *Escherichia coli* containing the plasmid pB6B15.23 (4). The plasmid encodes a 71,000 dalton fusion protein, the majority of which is the M-MuLV Reverse Transcriptase.

Applications:
- cDNA preparation, both first and second strand synthesis (3).
- DNA sequencing (5)

Reaction Buffer: 1X M-MuLV Reverse Transcriptase Buffer [50 mM Tris-HCl (pH 8.3), 8 mM $MgCl_2$, 10 mM dithiothreitol]. Supplement with dNTPs (not included). Incubate at 37°C.

Quality Assurance: M-MuLV Reverse Transcriptase is tested for its ability to synthesize full length cDNA from a 7.5 kb RNA. Each lot is also free of significant RNase and endonuclease activity.

Unit Definition: One unit is the amount of enzyme required to incorporate 1 nmole of TTP into an acid insoluble form in 10 minutes at 37°C using poly(rA)-oligo(dT) as template primer.

Unit Assay Conditions: 50 mM Tris-HCl (pH 8.3), 8 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM ^{3}H-TTP, 0.4 mM poly(rA)-oligo(d^T)12–18.

Concentration: 25,000 units/ml.

Storage Conditions: 50 mM Tris-HCl (pH 7.6), 1 mM EDTA, 5 mM dithiothreitol, 0.1% NP40, 0.1 M NaCl, and 50% glycerol. Store at –20 °C.

References:

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(3) Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, second edition, pp. 5.52–5.55, 8.11-8.17 Cold Spring Harbor Laboratory, Cold Spring Harbor.
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Cloned at NEBiolabs RR Recombinant Enzyme

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NEW

CyDye™ DIGE Fluor minimal dyes

ORDERING INFORMATION

Product	Quantity	Code Number	Price
CyDye DIGE Fluor, Cy2 minimal dye	10 nmol	25-8008-60	POR
CyDye DIGE Fluor, Cy3 minimal dye	10 nmol	25-8008-61	POR
CyDye DIGE Fluor, Cy5 minimal dye	10 nmol	25-8008-62	POR
CyDye DIGE Fluor, Cy2 minimal dye	25 nmol	RPK0272	POR
CyDye DIGE Fluor, Cy3 minimal dye	25 nmol	RPK0273	POR
CyDye DIGE Fluor, Cy5 minimal dye	25 nmol	RPK0275	POR

- ***Allows detection of up to three prelabelled protein samples and standards on the same 2-D electrophoresis gel.***
- Size- and charge-matched dyes enable co-migration of labelled samples within the gel.
- Bright and highly sensitive dyes allow the use of the minimal labelling technique.
- Minimal loss of signal during labelling, separation, and scanning.
- No change in signal over wide pH range used during first-dimension (IEF) separation.
- Discrete signal from each fluor with minimal cross-talk contributes to high accuracy.

Part of the office action

CyDye™ DIGE Fluor minimal dyes are exceptional dyes for multicolor analysis, offering bright and intense colors with narrow excitation and emission bands. The fluors are spectrally distinct, making them ideal for multicolor detection. CyDye™ DIGE Fluor minimal dyes utilize these benefits but are also specially developed to be size- and charge-matched specifically for 2-D DIGE using Ettan™ DIGE system.

Protein samples and the internal standard are each labelled with one CyDye™ DIGE Fluor minimal dye. These labelled samples are then combined, run on an isoelectric focusing gel in the first dimension, and separated by SDS-PAGE in the second dimension. Electrophoresis is simplified with Ettan™ IPGphor™, or Multiphor™ II with Immobiline™ DryStrip gels in the first dimension and Ettan™ DALT*twelve* or Ettan™ DALT*six* in the second dimension.

The ability to multiplex different CyDye™ DIGE Fluor dye-labelled samples on the same gel means that the different samples will be subject to exactly the same first- and second-dimension running conditions. Consequently, the same protein labelled with any of the CyDye™ DIGE Fluor minimal dyes and separated on the same gel will migrate to the same position on the 2-D gel and overlay. This limits experimental variation and ensures accurate within-gel matching.

CyDye™ DIGE Fluor minimal dyes have an NHS-ester reactive group, and are designed to covalently attach to the epsilon amino group of lysine of proteins via an amide linkage. The ratio of dye to protein has been specifically designed to ensure the dyes are limiting in the reaction. As a result, the dyes label approximately 3% of the available proteins and then only on a single lysine per protein (i.e. one dye per protein, or minimal labelling).

The amino acid lysine in proteins carries an intrinsic single positive charge at neutral or acidic pH. CyDye™ DIGE Fluor minimal dyes also carry a single positive charge which, when coupled to the lysine, replaces the single positive charge of the lysine with its own, ensuring that the pI of the protein does not significantly alter compared with the same unlabelled protein.

Part of the office action

Related Pr ducts	**C de Number**
DIGE Enabled Typhoon 9400 Variable Mode Imager	63-0055-79
DeCyder™ Differential Analysis Software	
Ettan™ DALT*twelve* Large Vertical System	